

Listing of Claims

The claim listing below replaces and/or supersedes all earlier claim listings.

1. (Canceled)

2. (Canceled)

5 3. (Currently Amended) An isolated aggrecan peptide fragment consisting of amino acids 1-40 of SEQ ID NO:1, wherein said fragment is capable of functioning as a substrate for an aggrecan degrading metallo protease (ADMP).

4. (Canceled)

10 5. (Currently Amended) An isolated aggrecan peptide fragment consisting of amino acids 1-40 of SEQ ID NO:2, wherein said fragment is capable of functioning as a substrate for an ADMP.

 6. (Currently Amended) An isolated aggrecan peptide fragment consisting of amino acids 1-40 of SEQ ID NO:3, wherein said fragment is capable of functioning as a substrate for an ADMP.

15 7. (Canceled)

 8. (Currently Amended) A peptide of claims 3, [[4]], 5, or 6 [[or 7]] wherein the peptide further comprises a ~~has a~~ linking moiety.

 9. (Previously Presented) A peptide of claim 8 wherein the linking-moiety is biotinylated or a biotinylated lysine.

20 10. (Currently Amended) A peptide of claim 8 wherein the linking-moiety comprises ~~contains~~ a chromophore.

11. (Currently Amended) A peptide of claim 8 wherein the linking moiety is peptide ~~has~~
a C-terminal linking-moiety.

12. (Currently Amended) A peptide of claim 8 wherein the linking moiety is peptide ~~has~~
a C-terminal ~~linking-moiety that is a~~ biotinylated lysine.

5 13. (Currently Amended) A peptide of claim 8 wherein the linking moiety is peptide ~~has~~
an N-terminal linking-moiety.

14. (Currently Amended) A peptide of claim 8 wherein the linking moiety is peptide ~~has~~
an N-terminal ~~linking-moiety that is a~~ biotinylated lysine.

15. (Currently Amended) An isolated proteolytic cleavage product, of the isolated
10 peptide fragment of claim 3 [[4,] 5, or 6, [[or 7,]] comprising the amino acids from the N-
terminus through P1 of the ADMP-susceptible cleavage bond, wherein said product functions as
an indicator of ADMP activity.

16. (Currently Amended) An isolated proteolytic cleavage product, of the isolated
peptide fragment of claim 3, [[4,] 5, or 6, [[or 7,]] comprising the amino acids from P1' of the
15 ADMP-susceptible cleavage bond through the C-terminus, wherein said product functions as an
indicator of ADMP activity.

17. (Previously Presented) A proteolytic cleavage product of claim 15 wherein the
peptide is biotinylated.

18. (Currently Amended) A proteolytic cleavage product peptide of claim 15 wherein the
20 peptide further comprises ~~has~~ an N-terminal linking-moiety.

19. (Currently Amended) A proteolytic cleavage product peptide of claim 16 wherein the
peptide further comprises ~~has~~ a C-terminal linking-moiety.

20. (Previously Presented) A proteolytic cleavage product peptide of claim 18 wherein the linking-moiety is a biotinylated lysine.

21. (Previously Presented) A proteolytic cleavage product peptide of claim 19 wherein the linking-moiety is a biotinylated lysine.

5 22. (Currently Amended) A proteolytic cleavage product peptide of claim 18 wherein the linking-moiety comprises ~~contains~~ a chromophore.

23. (Currently Amended) A proteolytic cleavage product peptide of claim 19 wherein the linking-moiety comprises ~~contains~~ a chromophore.

10 24. (Previously Presented) An isolated, C-terminal biotinylated, aggrecan peptide fragment comprising SEQ ID NO:5.

25. (Previously Presented) An isolated, N-terminal biotinylated, aggrecan peptide fragment comprising SEQ ID NO:6.

15 26. (Withdrawn) A method for the determination of the presence of aggrecan-degrading metalloprotease activity comprising: (a) binding an ADMP substrate peptide of claim 1 to a streptavidin-coated microtiter plate; (b) rinsing the microtiter plate with assay buffer; (c) incubating the microtiter plate with an ADMP-containing sample; (d) rinsing the microtiter plate; (e) incubating the microtiter plate with a neoepitope antibody solution; (f) rinsing the microtiter plate; (g) incubating microtiter plates with secondary-detection antibody solution; (h) incubating the microtiter plate with an appropriate substrate solution; (i) quenching the reaction; (j) reading
20 the optical density;

27. (Withdrawn) The method of claim 26, wherein said ADMP peptide substrate comprises a covalently-linked linking-moiety.

28. (Withdrawn) A method for the determination of ADMP activity by quantifying the appearance of a product peptide comprising: (a) incubating an ADMP substrate peptide of claim 1 with assay buffer and ADMP-containing sample; (b) quenching the reaction; (c) injecting a portion of the reaction mixture onto a reverse-phase HPLC column; (d) eluting the peptide with an organic solvent; (e) reading the absorbance; (f) determining the quantity based on a standard curve.

29. (Withdrawn) A method for assaying compounds for activity against an ADMP comprising: (a) providing an ADMP and an ADMP substrate; (b) contacting said ADMP with a candidate inhibitory compound in the presence of said ADMP; and (c) measuring the inhibition of the ADMP activity.

30. (Withdrawn) A method for assaying compounds according to claim 29 wherein the ADMP activity is monitored according to claim 26 or 28.

31. (Currently Amended) A peptide of claim 3, [[4,]] or 5 wherein the P1 amino acid residue, Glu, of the ADMP-sensitive Glu³⁷³-Ala³⁷⁴ bond, which corresponds to the bond between positions 20 and 21 of SEQ ID NO: 1 and SEQ ID NO: 2, is esterified.

32. (Currently Amended) A peptide of claim 3, [[4,]] or 5 wherein the P1 amino acid residue, Glu, of the ADMP-sensitive Glu³⁷³-Ala³⁷⁴ bond, which corresponds to amino acid at position 20 of SEQ ID NO: 1 and SEQ ID NO: 2, is replaced with a Gln amino acid residue.

33. (Withdrawn) An assay for detecting ADMP activity which comprises: (a) incubating a sample containing soluble ADMPs or aggrecanase activity with an aggrecan substrate; and (b) monitoring production of aggrecan fragments produced by specific cleavage at an ADMP-susceptible site using a neoepitope antibody to the new N-terminus or the new C-terminus

generated by specific ADMP-mediated cleavage by the Problot assay comprising: (1) incubate a polyvinyl-denedifluoride (PVDF) cationically charged membrane, secured in a wellled filtration plate, with a sample containing ADMP-degraded aggrecan; (2) wash any unbound aggrecan from the filtration plate; (3) couple any unreacted cationic sites on the PVDF membrane with a
5 solution of bovine serum albumin (BSA); (4) wash any unbound BSA from the filtration plate; (5) remove glycosaminoglycan side chains from the bound aggrecan with deglycosylation enzymes, wash membrane; (6) incubate PVDF membrane with a neoepitope antibody to fragments generated by cleavage at an ADMP-sensitive site, wash membrane; (7) incubate PVDF membrane with secondary detection antibody, wash membrane; (8) incubate PVDF
10 membrane with detection substrate; (9) drain solution into wellled plate, obtain absorbance readings on individual samples; compare values to those obtained for standard curve.

34.(Withdrawn) A method for assaying compounds according to claim 29 wherein ADMP activity is monitored according to claim 33.

35.(Withdrawn) An assay according to claim 33 wherein the tissue or cell source of
15 ADMPs is cartilage or chondrocytes.

36. (Withdrawn) An assay according to claim 33 or 34 wherein the aggrecan substrate is native aggrecan isolated from human or animal tissue.

37. (Withdrawn) An assay according to claim 33 or 34 wherein the aggrecan substrate is a recombinant aggrecan molecule or recombinant portion of the aggrecan molecule containing an
20 aggrecanase-sensitive cleavage site.

38. (Withdrawn) An assay according to claim 33 or 34 wherein the recombinant portion of the aggrecan molecule contains the E³⁷³⁻⁻⁻³⁷⁴ A bond.

39. (Withdrawn) An assay according to claim 33 or 34 wherein the recombinant aggrecan fragment contains the E¹⁵⁴⁵⁻⁻⁻¹⁵⁴⁶G bond.

40. (Withdrawn) An assay according to claim 33 or 34 wherein the portion of the aggrecan molecule contains the E¹⁷¹⁴⁻⁻⁻¹⁷¹⁵G bond.

5 41. (Withdrawn) An assay according to claim 33 or 34 wherein the recombinant portion of the aggrecan molecule contains the E¹⁸¹⁹⁻⁻⁻¹⁸²⁰A bond.

42. (Withdrawn) An assay according to claim 33 or 34 wherein the recombinant portion of the aggrecan molecule contains the E¹⁹¹⁹⁻⁻⁻¹⁹²⁰L bond.

10 43. (Withdrawn) A method according to claims 26, 30, 33, or 34 wherein the neoepitope antibody recognizes the new N-terminus or new C-terminus generated by cleavage at the E373 - A374 bond.

44. (Withdrawn) A method of any of claims 26, 30, 33, or 34 wherein the neoepitope antibody is the BC-3 monoclonal antibody.

15 45. (Withdrawn) A method of any of claims 26, 30, 33, or 34 wherein the neoepitope antibody recognizes the new N-terminus or new C-terminus generated by cleavage at the E1545- G1546 bond.

46. (Withdrawn) A method of any of claims 26, 30, 33, or 34 wherein the neoepitope antibody recognizes the new N-terminus or new C-terminus generated by cleavage at the E1714- G1715 bond.

20 47. (Withdrawn) A method of any of claims 26, 30, 33, or 34 wherein the neoepitope antibody recognizes the new N-terminus or new C-terminus generated by cleavage at the E1819- A1820 bond.

48. (Withdrawn) A method of any of claims 26, 30, 33, or 34 wherein the neoepitope antibody recognizes the new N-terminus or new C-terminus generated by cleavage at the E1919-L1920 bond.

5 49. (Withdrawn) A method of use of the assay in claim 33 for detecting ADMP-generated aggrecan fragments in culture media from tissue or cell cultures stimulated to induce aggrecanase-mediated degradation.

50. (Withdrawn) A method of use of the assay in claim 33 for detecting aggrecanase-generated aggrecan fragments in biological fluids, tissue extracts or homogenates, serum or urine from patients with aggrecanase-associated diseases.

10 51. (Withdrawn) A method for diagnosing arthritic diseases in a mammal by monitoring ADMP-generated aggrecan fragments according to claims 33.

52. (Withdrawn) A method for diagnosing a disease in a mammal characterized by overproduction or up-regulated production of an ADMP by monitoring fragments generated at an ADMP-sensitive site according to claims 33.

15 53. (Canceled)

54. (Currently Amended) A proteolytic cleavage product of claim 16 wherein the product peptide is biotinylated.